Hemoglobin Adducts as Biomarkers of Carcinogen Uptake

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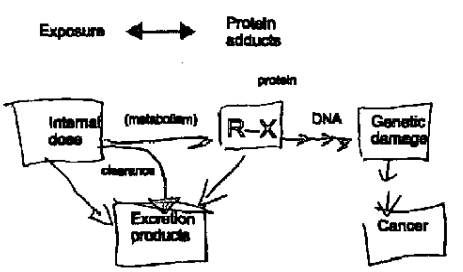
Adduct Dosimetry of Chemical Carcinogens



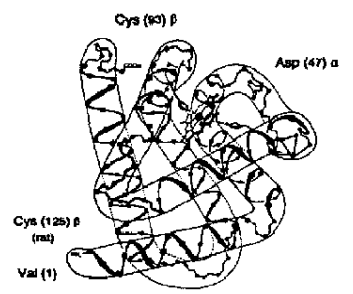
Exposure Assessment with Hemoglobin Adducts

- Long-term (0–4 months) integral dosimetry
- Adduct levels reflect bioavailable dose (not directly the internal exposure)
- Responsive to metabolic polymorphisms Based on how much that has been taken up

Chemical Carcinogen Exposures Detected by Protein Adduct Analysis

- Alkylating agents
  - *N*-Nitrosamines: NNK
  - Epoxides: PAH metabolites
- Carbonyl compounds
  - Lipid peroxidation products
  - Products of incomplete combustion
- Aromatic amines
  - *N*-hydroxylamine metabolites

Carcinogen Binding Sites in Hemoglobin



Quantitative Analysis of Protein Adducts

- Isolation and purification of protein
- Separation of adduct from protein
  - Proteolysis
  - Specific cleavage
- Instrumental analysis
  - High performance gas, liquid chromatography
  - Mass spectrometric, fluorescence detection

Quantitative Analysis of Aromatic Amine-Hemoglobin Adducts

- Preparation of hemoglobin solution
- Hydrolytic cleavage of adduct bond to protein
- Solvent extraction
- GC-MS analysis
  - Derivatization
  - Negative ion chemical ionization
  - Quantitation by isotope dilution mass spectrometry

Aromatic Amines, Smoking and Bladder Cancer

- 1895: First clinical report of bladder cancer-aromatic amine exposure association
- 1938: Demonstration of 2-naphthylamine carcinogenicity in dogs
- 1953: Epidemiological evidence for human bladder carcinogenicity of 4-aminobiphenyl
- 1967: Detection of aromatic amines in cigarette smoke
- 1971: Aromatic amines hypothesized to be etiological agents for tobacco-related bladder cancer
- 1979: *N*-Acetyltransferase phenotype shown to influence bladder cancer risk associated with smoking

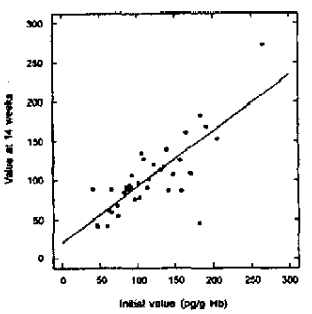
Aromatic Amines Present in Tobacco Smoke

Structure	Abundance (ng/cig)
Aniline	100–1,000
2-, 3-, 4-Methylanilines	50–300
2,- 3-, 4-Ethylanilines	10–50
2,3- 2,4-, 2,5-, 2,6-Dimethylanilines	25–150
1-, 2-Naphthylamines	1–10
2-, 3-, 4-Aminobiphenyls	1–10

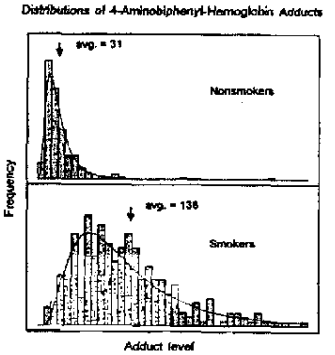
Molecular Epidemiology Studies of Aromatic Amine-Hemoglobin Adducts

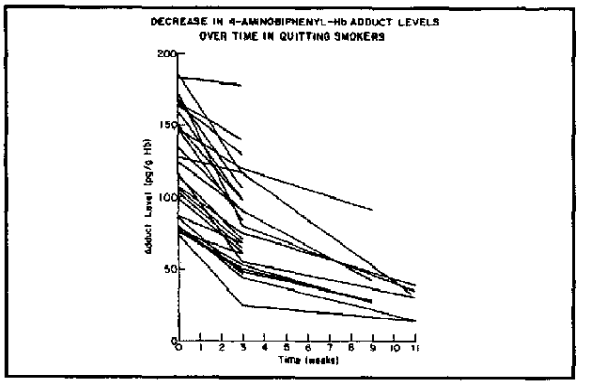
- Role of smoking in exposure
  - Comparison of smokers with nonsmokers
  - Effect of smoking withdrawal
  - Environmental tobacco smoke
- Effect of genetic polymorphisms
  - NAT1, NAT2 phenotypes
  - *GST* genotypes
- Association with risk of bladder cancer
  - Cross-sectional studies
  - Case-control studies

Within-Person Variability of 4-Aminobiphenyl Hemoglobin Adducts



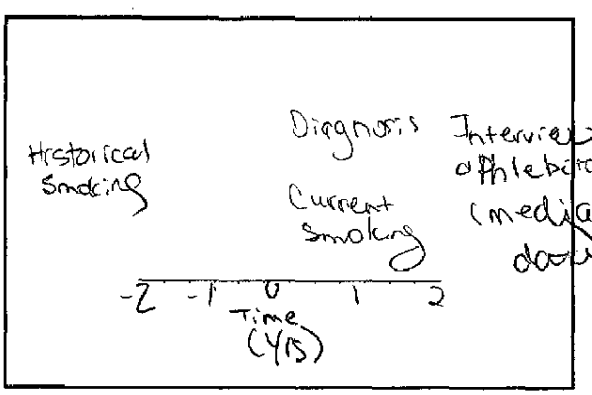
Distributions of 4-Aminobiphenyl-Hemoglobin Adducts





1992-1999 Case-Control Study of Bladder Cancer in Los Angeles County

- Multiple aims – Smoking, Metabolic genotypes and phenotypes, Nutrition, Medicines, Hair dye
- ~~Arylamine exposure assessment~~ by hemoglobin adduct determination
- In-person interviews – Smoking/diet/analgesic use, ~~blood collection~~, overnight urine after caffeine dosing
- ~~1044 Cases – 79% Males, 88% non-Hispanic whites, mean age 56 yrs.~~ Blood/urine from 73% of cases
- 879 Controls – Individually matched to cases by age (± 5 yrs), sex, race, and neighborhood of residence at cancer diagnosis. Blood/urine from 79% of controls



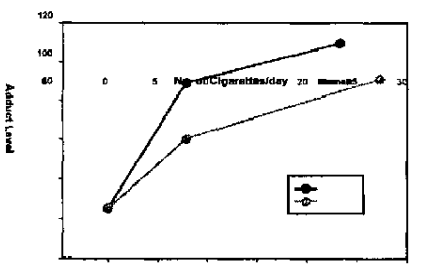
Lifetime Smoking History

Geometric mean levels of 4-ABP-hemoglobin adducts (pg/g Hb) among bladder cancer patients and control subjects.[a]

	Cases	Controls	2-sided P (case-control diff.)
Total subjects[b]	38.8	28.7	<0.0001
Nonsmokers	28.8	22.1	<0.0001
Smokers[b]	94.1	67.3	<0.0001
Further adjusted for other risk factors[c]			
Total subjects	36.2	29.2	<0.0001

[a]Age and sex were adjusted for in all runs.
[b]Further adjusted for average number of cigarettes/day within 60 days of blood draw.
[c]The other risk factors were: lifetime smoking history, NAT2 phenotype, *GSTM1* genotype, use of non-steroidal anti-inflammatory drugs, and dietary carotenoid intake.

Gender Difference in Dose-Response



Geometric Means vs.

Case-Control Study: Main Findings

- 4-Aminobiphenyl hemoglobin adducts strongly associated with risk of bladder cancer
- Risk also associated with non-tobacco-related exposure to 4-aminobiphenyl
- Other amine adducts associated with risk independently of smoking

Summary

- Hemoglobin adducts provide precise exposure assessment for the period 0-4 months prior to sample collection
- Tobacco smoke is the major source of non-occupational aromatic amine exposure
- Significant other environmental sources of aromatic amine exposure exist
- Aromatic amine exposure from all sources may be a major cause of human bladder cancer

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Notes:

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Peter Shields, MD

Notes:

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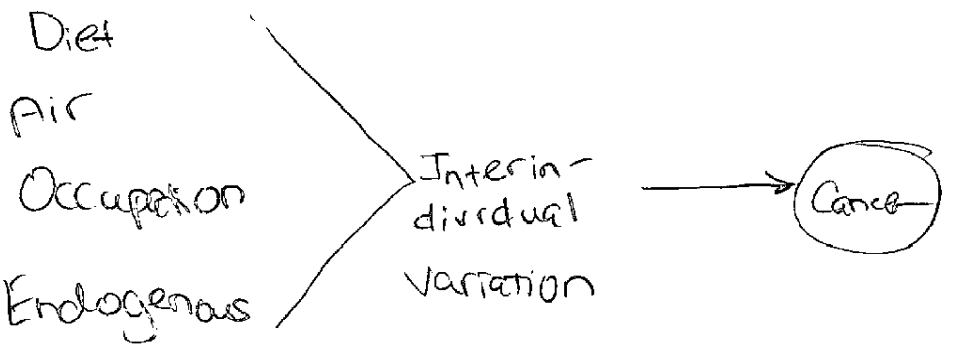
Exposure → Effect

External Exp. | Internal Dose | Biologically Effective Dose

Cancer Epidemiology: A New Paradigm

New



Old